

Calculation of normalized phosphate and carbonate concentrations for precipitation

Assume $[Ca^{2+}]$, $[Fe^{3+}]$, $[Ba^{2+}]$, and $[Ga^{3+}]$ are at unit concentration of 1 M

For $Ca_3(PO_4)_2 \rightleftharpoons 3Ca^{2+} + 2PO_4^{3-}$ where $K_{sp} = [Ca^{2+}]^3[PO_4^{3-}]^2$ and $K_{sp} = 2.07 \text{ E-33}$

then $2.07 \text{ E-33} = [Ca^{2+}]^3[PO_4^{3-}]^2$

$$2.07 \text{ E-33} = (1)^3[PO_4^{3-}]^2$$

$$2.07 \text{ E-33} = [PO_4^{3-}]^2$$

for Ca^{2+} $[PO_4^{3-}] = 4.5 \text{ E-17 M}$

Similarly, for $FePO_4 \rightleftharpoons Fe^{3+} + PO_4^{3-}$ where $K_{sp} = [Fe^{3+}][PO_4^{3-}]$ and $K_{sp} = 9.91 \text{ E-16}$

then $9.91 \text{ E-16} = [Fe^{3+}][PO_4^{3-}]$

$$9.91 \text{ E-16} = (1)[PO_4^{3-}]$$

for Fe^{3+} $[PO_4^{3-}] = 9.91 \text{ E-16 M}$

and for $Ba_3(PO_4)_2 \rightleftharpoons 3Ba^{2+} + 2PO_4^{3-}$ where $K_{sp} = [Ba^{2+}]^3[PO_4^{3-}]^2$ and $K_{sp} = 3.4 \text{ E-23}$

then $3.4 \text{ E-23} = [Ba^{2+}]^3[PO_4^{3-}]^2$

$$3.4 \text{ E-23} = (1)^3[PO_4^{3-}]^2$$

$$3.4 \text{ E-23} = [PO_4^{3-}]^2$$

for Ba^{2+} $[PO_4^{3-}] = 5.8 \text{ E-12 M}$

and for $GaPO_4 \rightleftharpoons Ga^{3+} + PO_4^{3-}$ where $K_{sp} = [Ga^{3+}][PO_4^{3-}]$ and $K_{sp} = 1 \text{ E-21}$

then $1 \text{ E-21} = [Ga^{3+}][PO_4^{3-}]$

$$1 \text{ E-21} = (1)[PO_4^{3-}]$$

for Ga^{3+} $[PO_4^{3-}] = 1 \text{ E-21 M}$

The strength of phosphate binding to Ca^{2+} and Fe^{3+} can be compared by dividing the phosphate concentration required for precipitation with Fe^{3+} by that required with Ca^{2+} :

$$(9.91 \text{ E-16}/4.5\text{E-17}) = 22$$

Thus, with the same amount of metal present, calcium should bind phosphopeptides 22 times more tightly than iron (III).

Calculations can also be performed to determine the $[\text{CO}_3^{2-}]$ required for precipitation of the carbonate salts of Ca^{2+} and Ba^{2+} when assumed to be 1 M:

For $\text{CaCO}_3 \leftrightarrow \text{Ca}^{2+} + \text{CO}_3^{2-}$ where $K_{\text{sp}} = [\text{Ca}^{2+}][\text{CO}_3^{2-}]$ and $K_{\text{sp}} = 3.36 \text{ E-9}$

then $3.36 \text{ E-9} = [\text{Ca}^{2+}][\text{CO}_3^{2-}]$

$$3.36 \text{ E-9} = (1)[\text{CO}_3^{2-}]$$

$$3.36 \text{ E-9} = [\text{CO}_3^{2-}]$$

$$[\text{CO}_3^{2-}] = 3.36 \text{ E-9 M}$$

and for $\text{FeCO}_3 \leftrightarrow \text{Fe}^{2+} + \text{CO}_3^{2-}$ where $K_{\text{sp}} = [\text{Fe}^{2+}][\text{CO}_3^{2-}]$ and $K_{\text{sp}} = 3.13 \text{ E-11}$

then $3.13 \text{ E-11} = [\text{Fe}^{2+}][\text{CO}_3^{2-}]$

$$3.13 \text{ E-11} = (1)[\text{CO}_3^{2-}]$$

$$3.13 \text{ E-11} = [\text{CO}_3^{2-}]$$

$$[\text{CO}_3^{2-}] = 3.13 \text{ E-11 M}$$

and for $\text{BaCO}_3 \leftrightarrow \text{Ba}^{2+} + \text{CO}_3^{2-}$ where $K_{\text{sp}} = [\text{Ba}^{2+}][\text{CO}_3^{2-}]$ and $K_{\text{sp}} = 2.58 \text{ E-9}$

then $2.58 \text{ E-9} = [\text{Ba}^{2+}][\text{CO}_3^{2-}]$

$$2.58 \text{ E-9} = (1)[\text{CO}_3^{2-}]$$

$$2.58 \text{ E-9} = [\text{CO}_3^{2-}]$$

$$[\text{CO}_3^{2-}] = 2.58 \text{ E-9 M}$$

The selectivity of calcium, iron, and barium for phosphate over carbonate can be calculated as follows:

Selectivity (S) = $[\text{CO}_3^{2-}] / [\text{PO}_4^{3-}]$ where $[\text{PO}_4^{3-}]$ and $[\text{CO}_3^{2-}]$ are the concentrations calculated above which represent the concentrations required for precipitation or complexation as a salt when the metal is assumed to be present at 1 M for normalized comparison.

$$S_{\text{calcium}} = 3.36 \text{ E-9} / 4.5 \text{ E-17}$$

$$= 7.5 \text{ E7}$$

$$S_{\text{iron}} = 3.13 \text{ E-11} / 9.91 \text{ E-16}$$

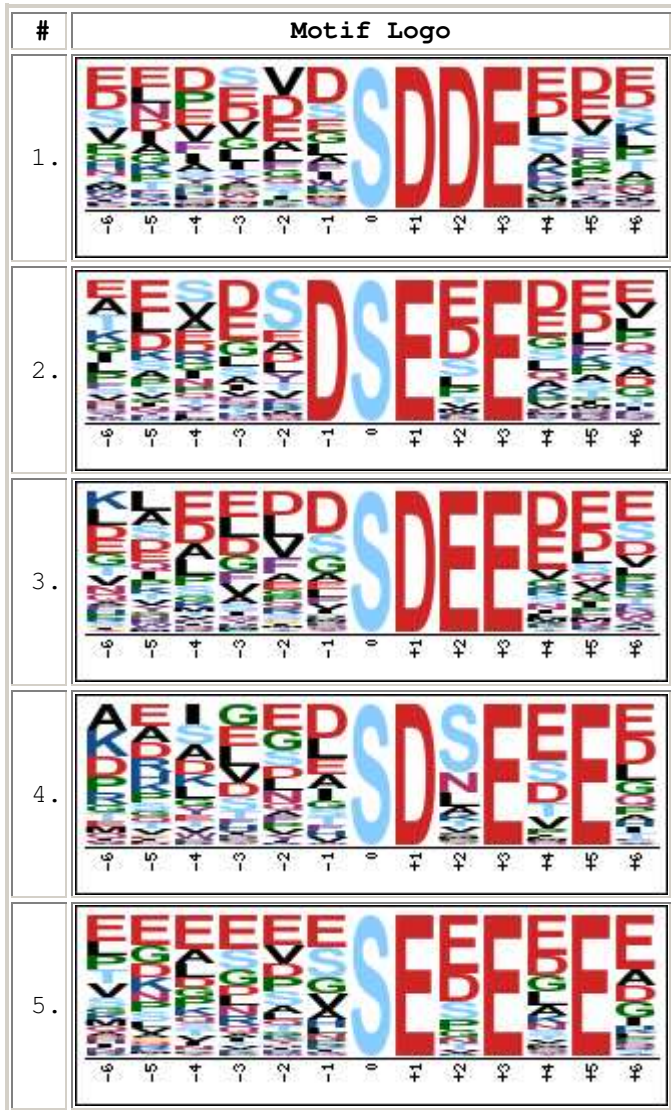
$$= 3.16 \text{ E4}$$

$$S_{\text{barium}} = 2.58 \text{ E-9} / 3.4 \text{ E-23}$$

$$= 7.6 \text{ E13}$$

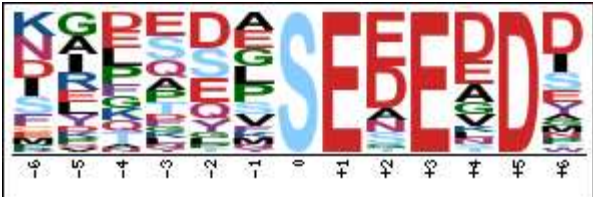
Thus, a phosphopeptide should bind hydroxyapatite nearly 100 million times more tightly than an unmodified peptide. Similarly, a phosphopeptide should bind a barium chromatograph resin nearly 100 trillion times more tightly than an unmodified peptide.

HAP Serine Motifs

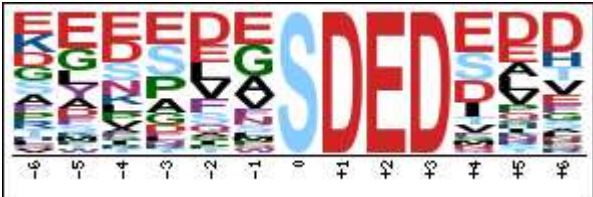


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1.	<u>.....sDDE...</u>	44.36	59	2342	340	1078751	79.93
2.	<u>.....DsE.E...</u>	44.69	59	2283	591	1078411	47.16
3.	<u>.....sDEE...</u>	41.25	66	2224	580	1077820	55.15
4.	<u>.....sD.E.E.</u>	47.65	46	2158	448	1077240	51.26
5.	<u>.....sE.E.E.</u>	43.10	58	2112	801	1076792	36.92
6.	<u>.....sP..E..</u>	30.91	76	2054	4295	1075991	9.27
7.	<u>.....s.DEE..</u>	44.79	30	1978	344	1071696	47.25
8.	<u>.....sD.E...</u>	32.00	111	1948	3209	1071352	19.02
9.	<u>.....sPE....</u>	27.20	71	1837	5168	1068143	7.99
10.	<u>.....DsD.D...</u>	44.94	50	1766	412	1062975	73.05
11.	<u>.....P.sP.....</u>	25.07	79	1716	7682	1062563	6.37
12.	<u>.....sE.E.D.</u>	39.16	24	1637	343	1054881	45.09
13.	<u>.....sDED...</u>	41.88	32	1613	326	1054538	64.17
14.	<u>.....sE.E...</u>	32.00	74	1581	4484	1054212	11.00
15.	<u>.....s.DDE..</u>	38.35	20	1507	215	1049728	64.80
16.	<u>.....sP.....</u>	16.00	266	1487	60203	1049513	3.12
17.	<u>.....s.D.D..</u>	32.00	56	1221	2758	989310	16.45
18.	<u>.....sE.D...</u>	32.00	61	1165	3358	986552	15.38
19.	<u>.....Ds...E..</u>	31.95	48	1104	3413	983194	12.52
20.	<u>.....s.E..E.</u>	25.27	42	1056	4814	979781	8.09
21.	<u>.....s.DE...</u>	26.43	31	1014	2017	974967	14.78
22.	<u>.....s...E.D</u>	28.95	34	983	2739	972950	12.29
23.	<u>.....sD.D...</u>	32.00	47	949	2604	970211	18.45
24.	<u>.....s.E..D.</u>	24.33	28	902	3156	967607	9.52
25.	<u>...D..sD.....</u>	24.61	25	874	2045	964451	13.49
26.	<u>.....s...E.E</u>	28.91	38	849	4597	962406	9.37
27.	<u>.....s...D.D</u>	23.00	24	811	2368	957809	11.97
28.	<u>.....s.D..D.</u>	23.81	22	787	2018	955441	13.24

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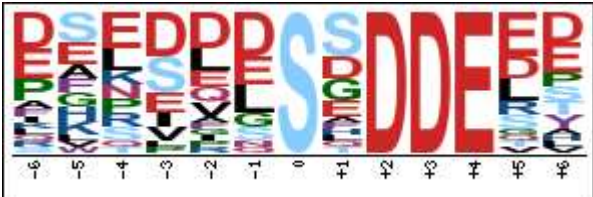
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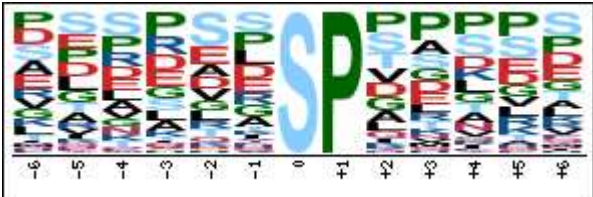
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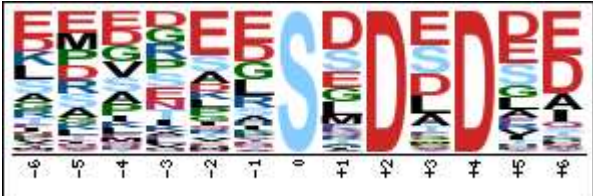
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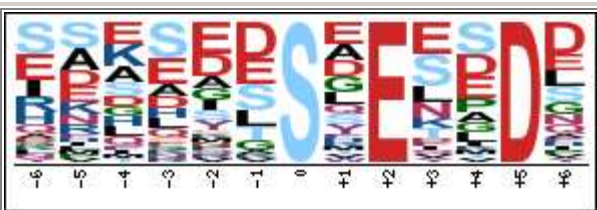
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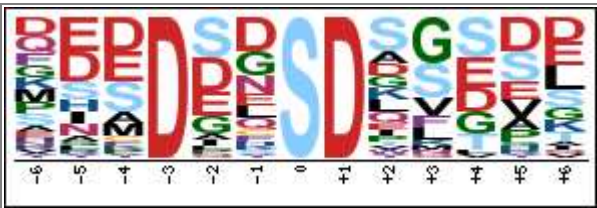
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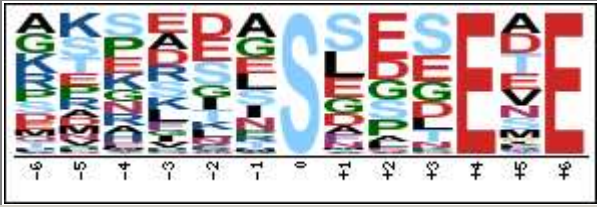
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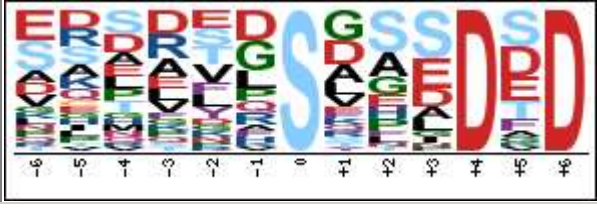
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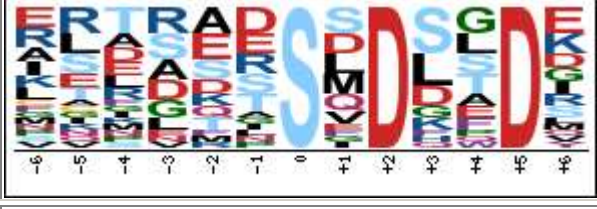
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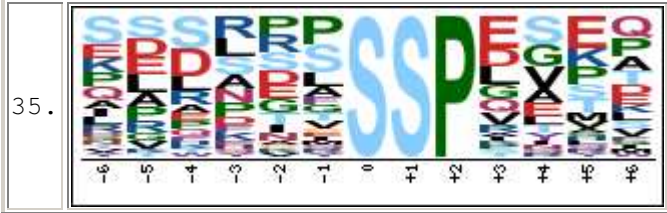
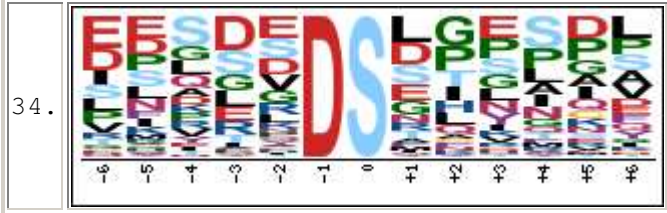
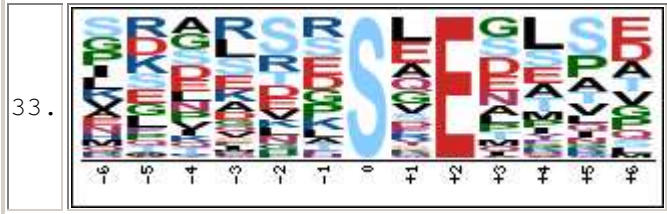
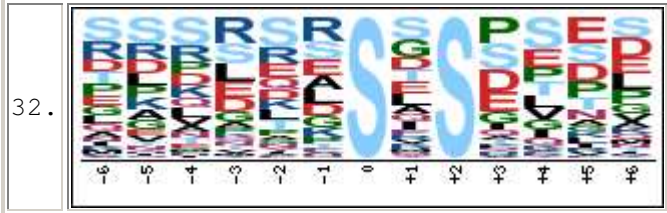
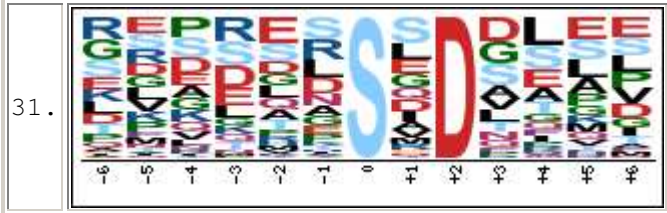
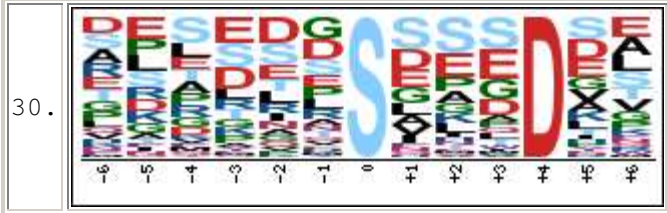


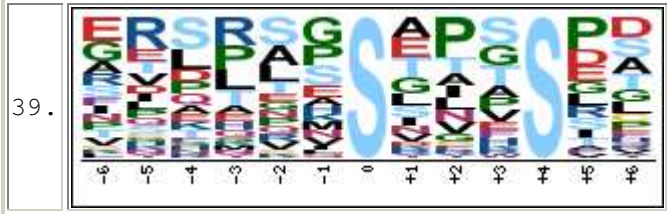
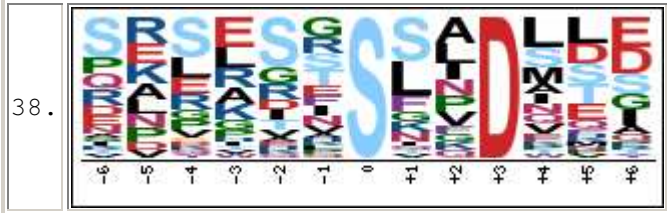
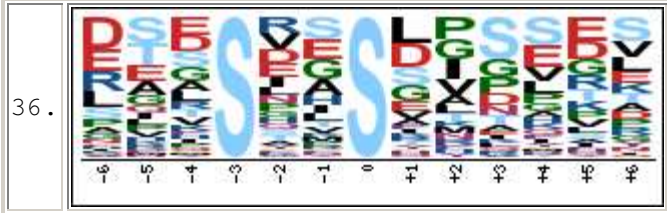
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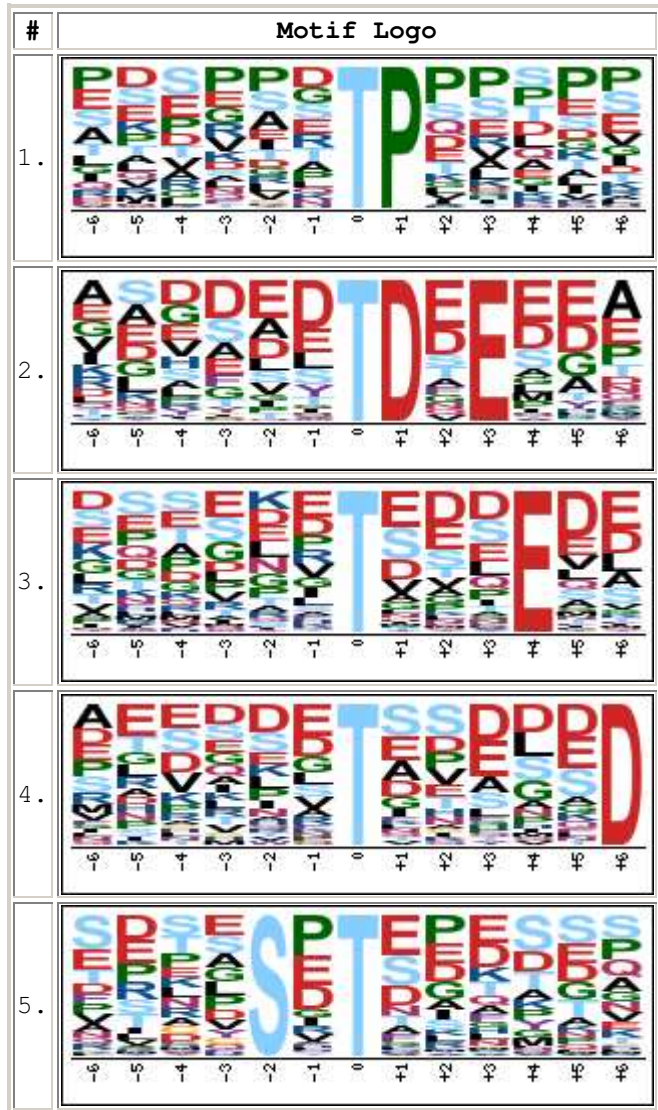
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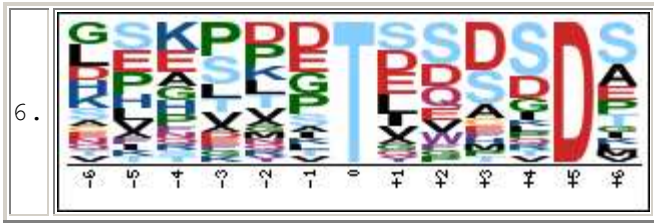




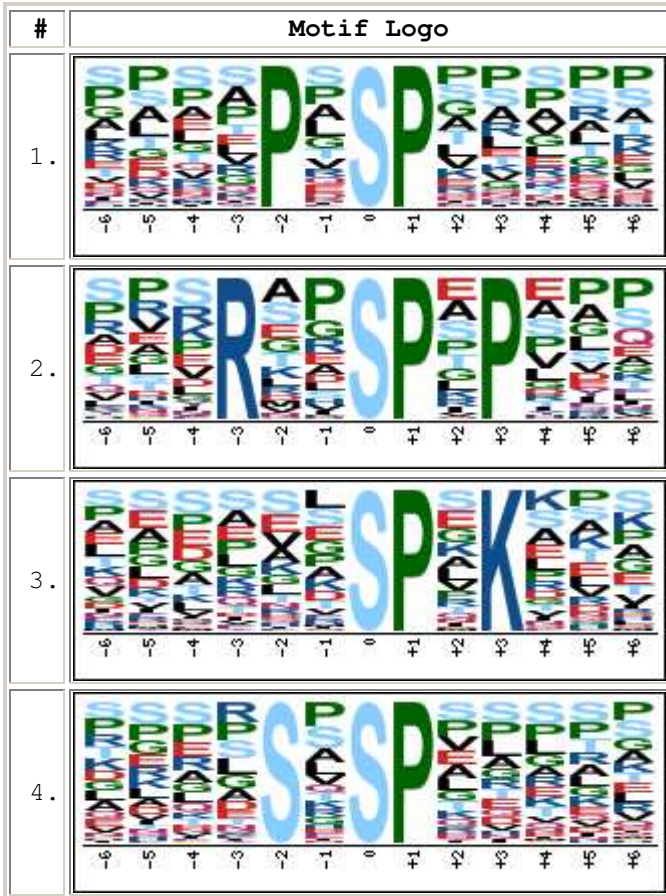
HAP Threonine Motifs



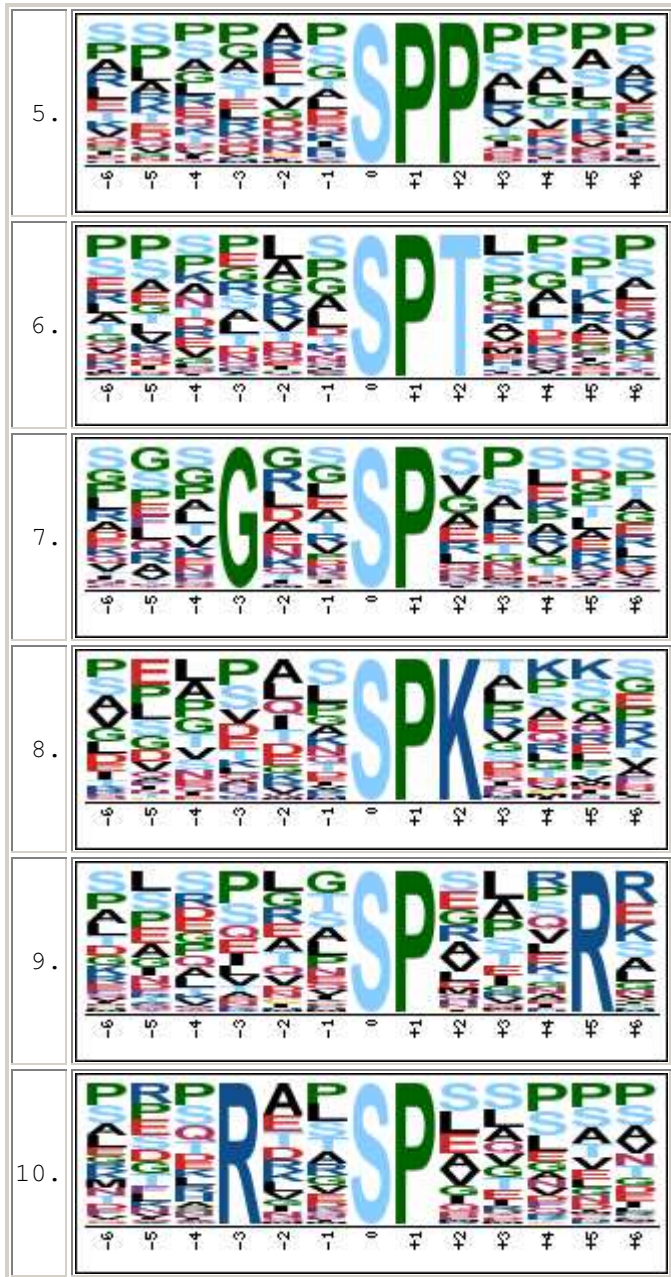
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1.tP.....	16.00	89	378	44989	683108	3.58
2.tD.E...	32.00	38	289	2347	638119	35.75
3.t...E..	16.00	57	251	40845	635772	3.53
4.t.....D	11.70	35	194	26510	594927	4.05
5.S.t.....	10.60	43	159	50547	568417	3.04
6.t....D.	7.33	21	116	23143	517870	4.05



HILIC-IMAC Serine Motifs

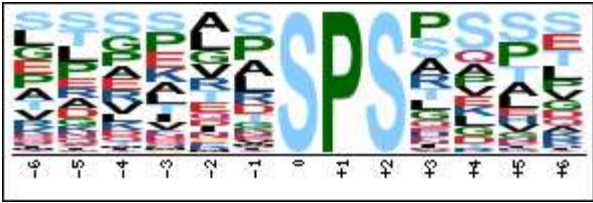


#	Motif	Motif Score	Foreground Matches	Foreground Size	Background Matches	Background Size	Fold Increase
1.	<u>....P.sP.....</u>	32.00	580	7754	8594	1078751	9.39
2.	<u>...R..sP.P...</u>	42.47	72	7174	514	1070157	20.90
3.	<u>.....sP.K...</u>	25.67	173	7102	3018	1069643	8.63
4.	<u>....S.sP.....</u>	25.13	383	6929	8427	1066625	7.00
5.	<u>.....sPP.....</u>	24.80	213	6546	4410	1058198	7.81
6.	<u>.....sPT.....</u>	24.83	156	6333	3108	1053788	8.35
7.	<u>...G..sP.....</u>	22.05	154	6177	3546	1050680	7.39
8.	<u>.....sPK.....</u>	22.51	105	6023	2226	1047134	8.20
9.	<u>.....sP...R.</u>	22.19	115	5918	2638	1044908	7.70
10.	<u>...R..sP.....</u>	23.37	106	5803	2339	1042270	8.14
11.	<u>.....sPS.....</u>	32.00	170	5697	5150	1039931	6.03
12.	<u>...RS.s.....</u>	32.00	168	5527	6844	1034781	4.60
13.	<u>...S..sP.....</u>	32.00	107	5359	3912	1027937	5.25
14.	<u>..S...sP.....</u>	32.00	79	5252	3137	1024025	4.91
15.	<u>.....Rs.SP...</u>	39.37	31	5173	585	1020888	10.46
16.	<u>...R..s..S...</u>	32.00	103	5142	5569	1020303	3.67
17.	<u>....SPs.....</u>	32.00	155	5039	7187	1014734	4.34
18.	<u>.....sD.E...</u>	32.00	196	4884	4531	1007547	8.92
19.	<u>.....PsSP.....</u>	39.56	53	4688	926	1003016	12.25
20.	<u>...R..s.S...</u>	32.00	91	4635	4844	1002090	4.06
21.	<u>...SP.s.....</u>	27.10	99	4544	7638	997246	2.84
22.	<u>.....sP.....</u>	16.00	605	4445	26329	989608	5.12

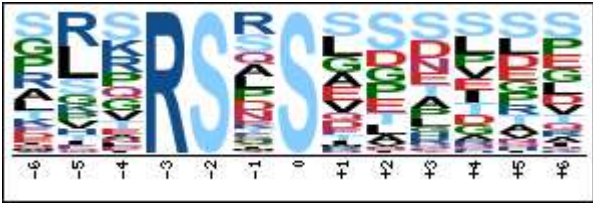


23.	<u>.....sSP....</u>	32.00	171	3840	7692	963279	5.58
24.	<u>..SP..s.....</u>	23.78	81	3669	7399	955587	2.85
25.	<u>..R..S.s.....</u>	23.96	68	3588	5403	948188	3.33
26.	<u>.....s.SP....</u>	26.29	77	3520	6194	942785	3.33
27.	<u>R..S..s.....</u>	26.14	71	3443	5796	936591	3.33
28.	<u>....SDsE.....</u>	30.82	24	3372	663	930795	9.99
29.	<u>.....sEEE....</u>	40.26	52	3348	1047	930132	13.80
30.	<u>.....s.DE....</u>	32.00	73	3296	2928	929085	7.03
31.	<u>.....s.ED....</u>	29.16	73	3223	4373	926157	4.80
32.	<u>.....s..SP..</u>	22.64	63	3150	6330	921784	2.91
33.	<u>...RR.s.....</u>	29.09	78	3087	3620	915454	6.39
34.	<u>.....s...SP.</u>	28.10	80	3009	6990	911834	3.47
35.	<u>..R.R..s.....</u>	28.06	57	2929	2727	904844	6.46
36.	<u>.....sD.D....</u>	27.96	52	2872	2850	902117	5.73
37.	<u>.....s.D....</u>	16.00	233	2820	40079	899267	1.85
38.	<u>....Rs.S....</u>	25.21	56	2587	4903	859188	3.79
39.	<u>.....s..E.E.</u>	26.54	62	2531	4875	854285	4.29
40.	<u>.....s.E....</u>	16.00	260	2469	52209	849410	1.71
41.	<u>....Gs.....</u>	15.35	263	2209	56624	797201	1.68
42.	<u>....PsTP....</u>	35.84	23	1946	390	740577	22.44
43.	<u>...R..s.P....</u>	19.46	29	1923	2096	740187	5.33
44.	<u>.SP...s.....</u>	20.64	49	1894	5361	738091	3.56
45.	<u>..S...s.....</u>	12.23	278	1845	71718	732730	1.54
46.	<u>..R...s.....</u>	9.53	159	1567	40036	661012	1.68
47.	<u>.....s..D....</u>	7.99	113	1408	28342	620976	1.76
48.	<u>.....s..E....</u>	7.78	130	1295	35759	592634	1.66
49.	<u>.....s.TP....</u>	15.69	30	1165	2808	556875	5.11
50.	<u>.....s..S....</u>	7.84	181	1135	58327	554067	1.51
51.	<u>....R.s.....</u>	6.31	84	954	24651	495740	1.77

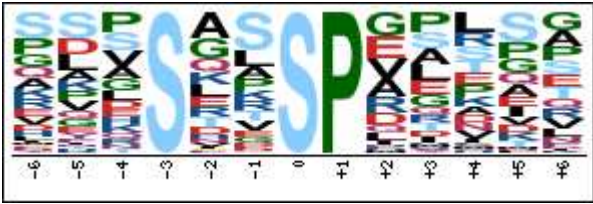
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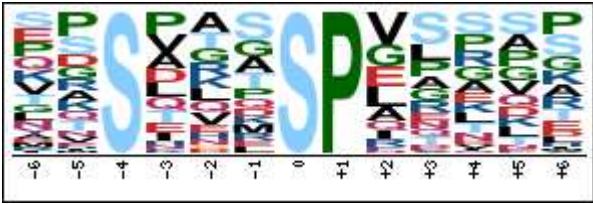
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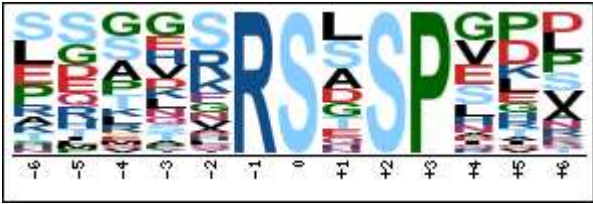
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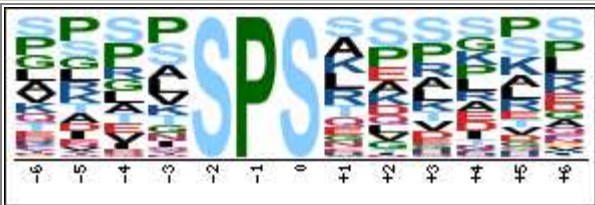
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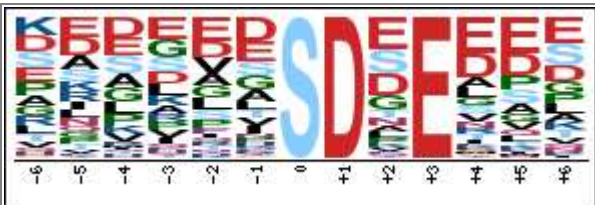
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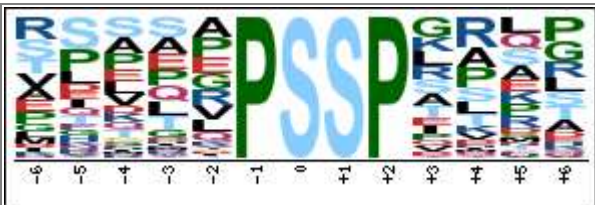
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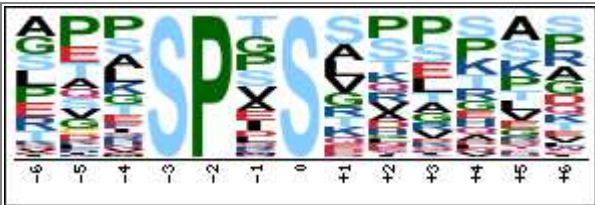
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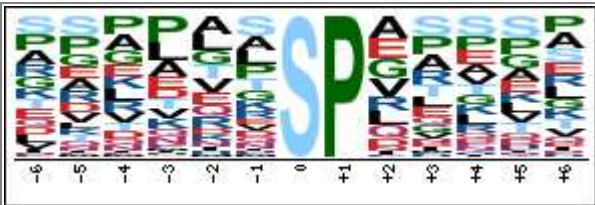
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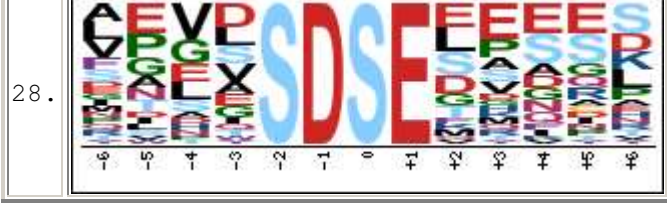
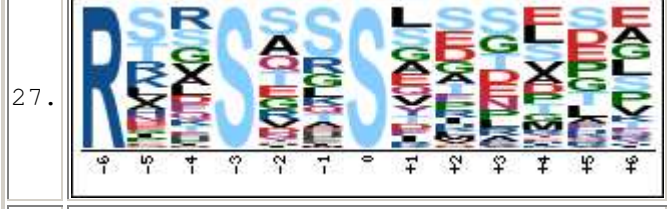
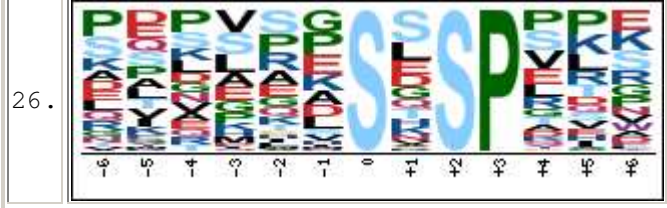
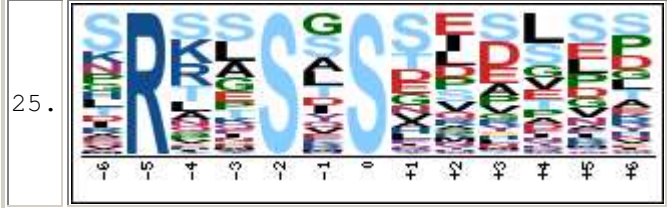
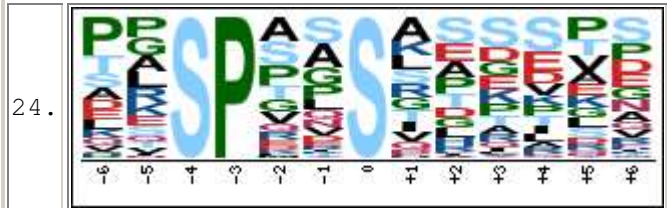
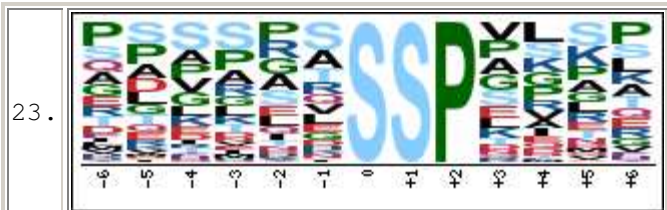


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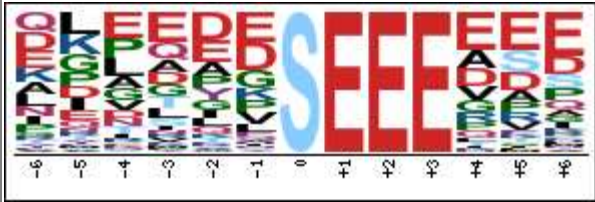


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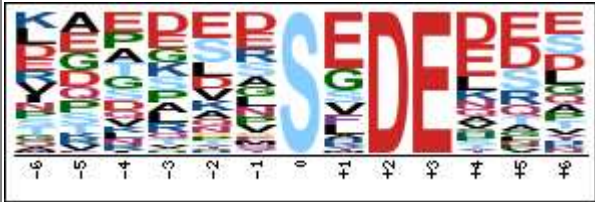




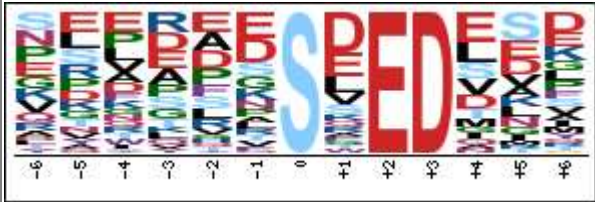
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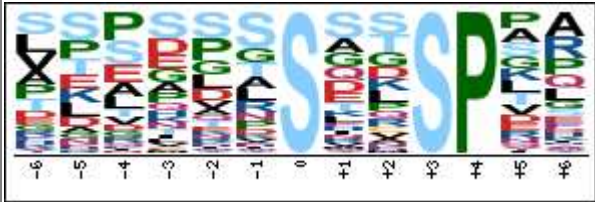
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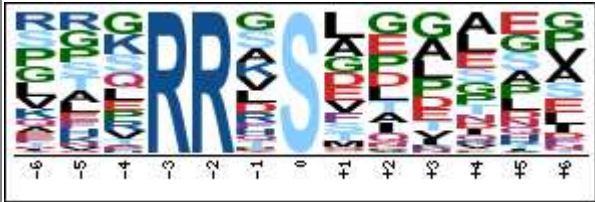
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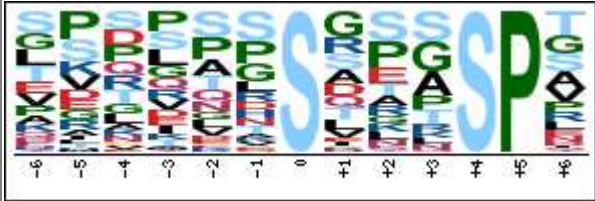
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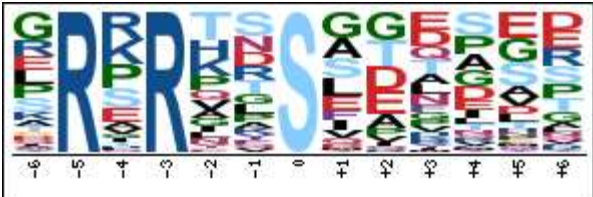
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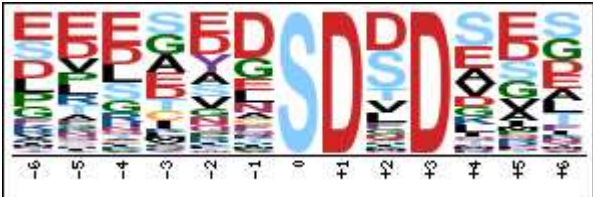
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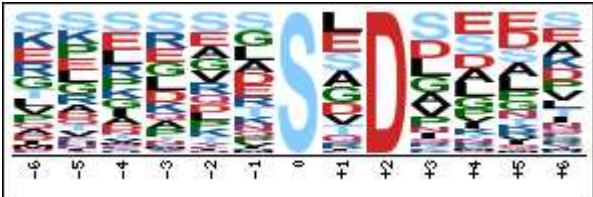
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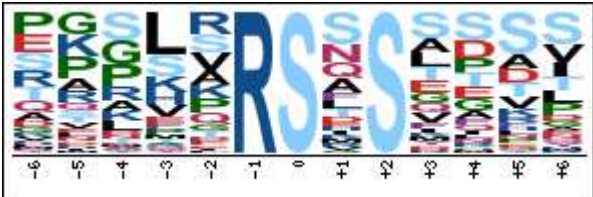
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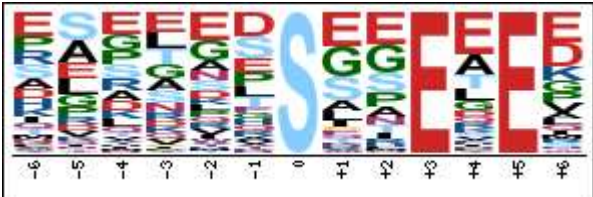
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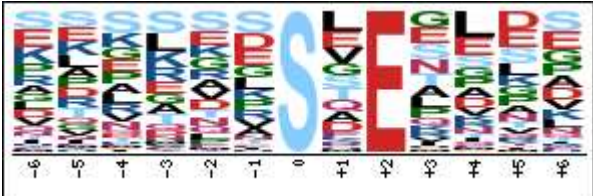
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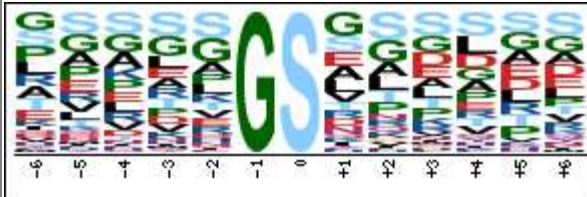
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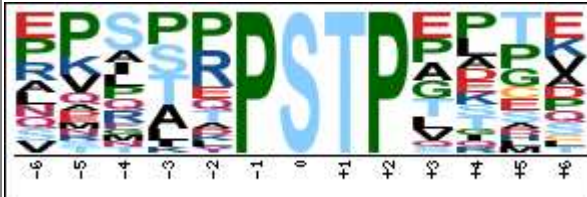
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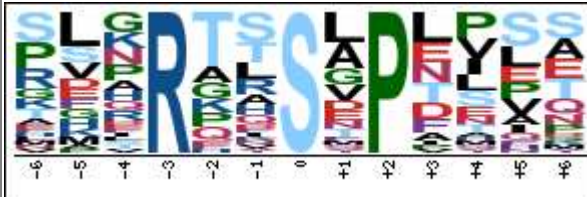
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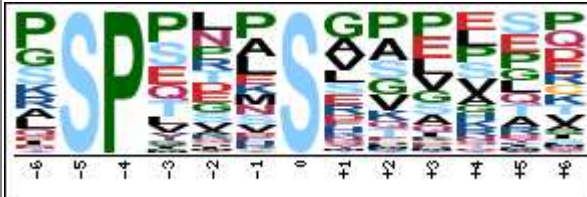
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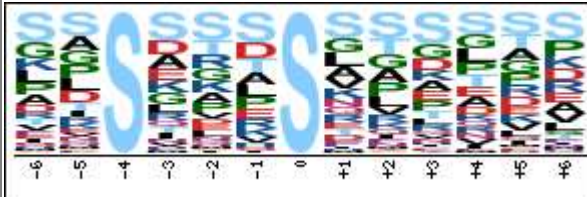
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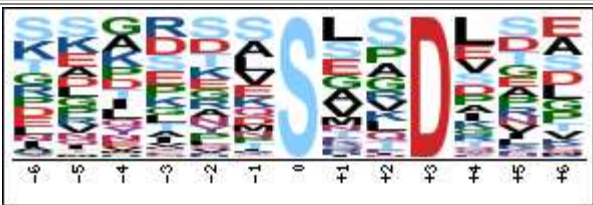
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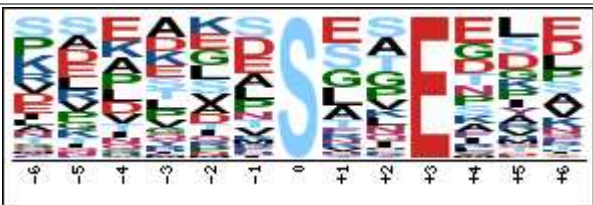
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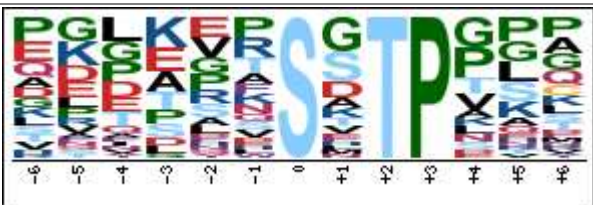
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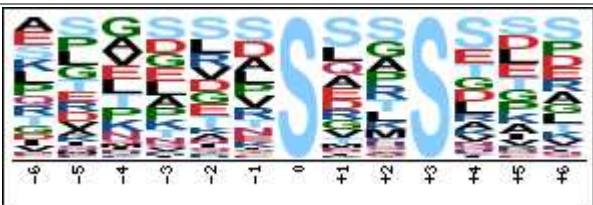
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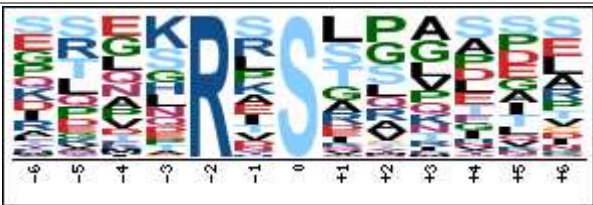
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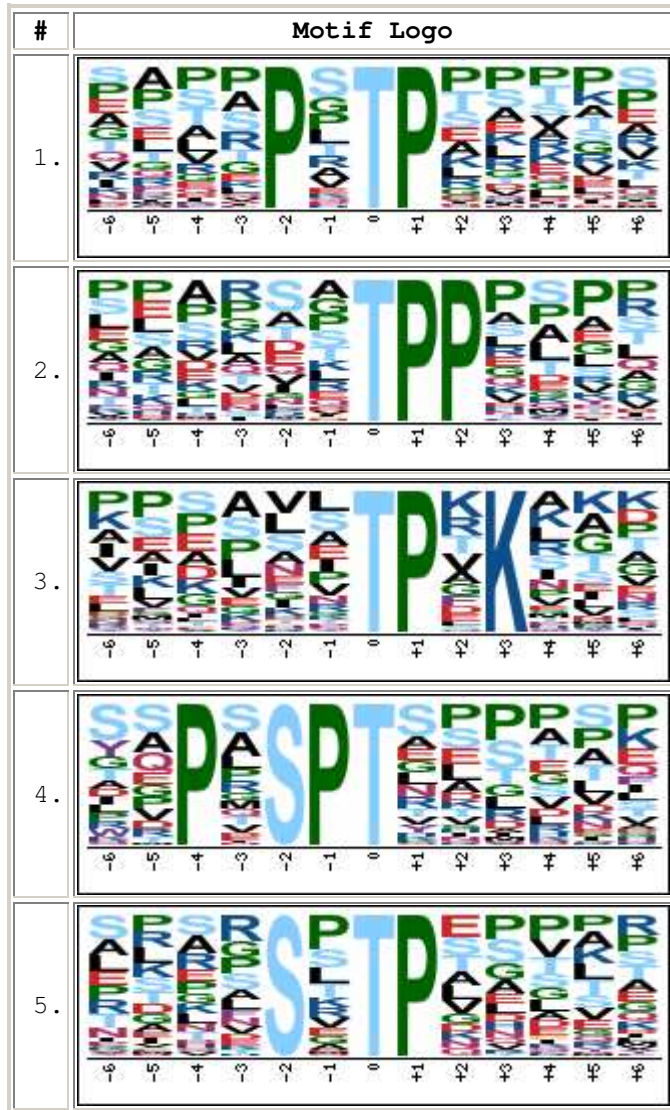
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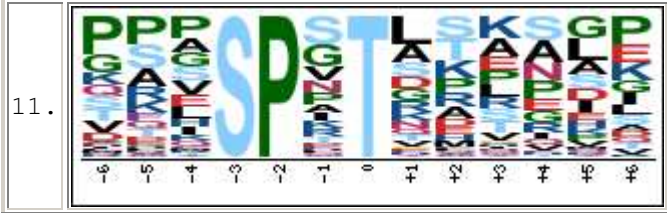
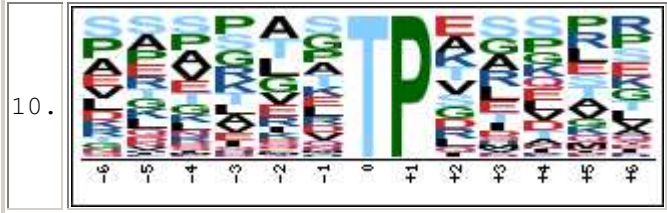
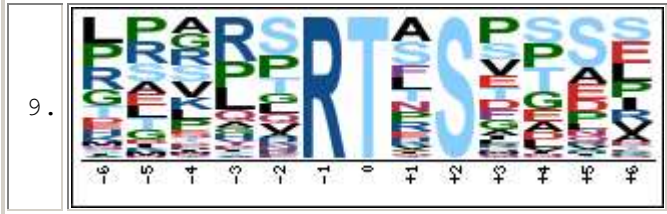
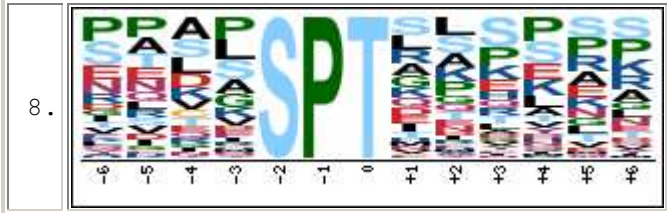
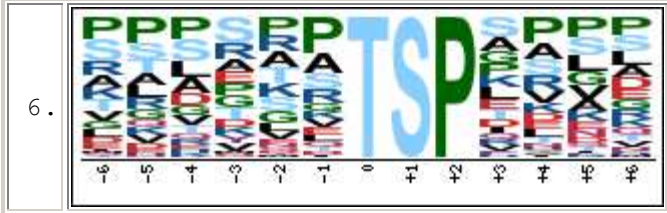
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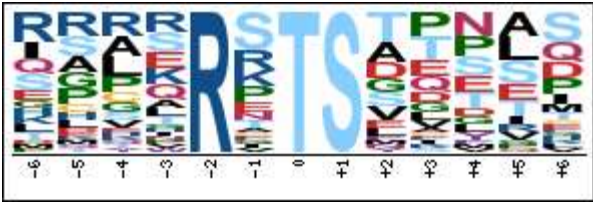
HILIC-IMAC Threonine Motifs



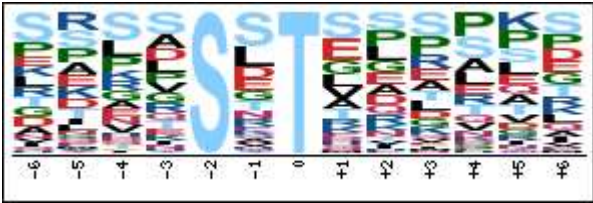
#	Motif	Motif Score	Foreground Matches	Foreground Size	Background Matches	Background Size	Fold Increase
1.	<u>....P.tP.....</u>	32.00	181	2146	4807	683108	11.99
2.	<u>.....tPP....</u>	32.00	113	1965	3182	678301	12.26
3.	<u>.....tP.K...</u>	24.71	52	1852	1866	675119	10.16
4.	<u>..P.SPt.....</u>	38.59	36	1800	511	673253	26.35
5.	<u>....S.tP.....</u>	24.75	79	1764	3857	672742	7.81
6.	<u>.....tSP....</u>	32.00	109	1685	4382	668885	9.87
7.	<u>.....tP.P...</u>	22.71	55	1576	2959	664503	7.84
8.	<u>....SPt.....</u>	32.00	65	1521	3317	661544	8.52
9.	<u>.....Rt.S....</u>	29.92	49	1456	3555	658227	6.23
10.	<u>.....tP.....</u>	16.00	223	1407	28097	654672	3.69
11.	<u>...SP.t.....</u>	25.43	41	1184	3906	626575	5.55
12.	<u>....R.tS.....</u>	23.19	30	1143	2712	622669	6.03
13.	<u>....S.t.....</u>	14.75	174	1113	51862	619957	1.87
14.	<u>.....t.SP...</u>	21.56	37	939	3396	568095	6.59
15.	<u>..S...t.....</u>	8.17	131	902	49119	564699	1.67
16.	<u>.....St.....</u>	6.34	103	771	41683	515580	1.65



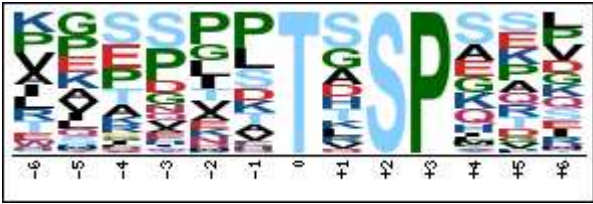
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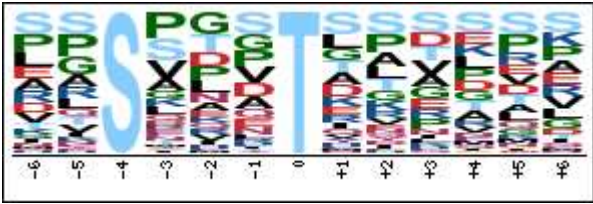
13.



14.



15.



16.

